



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁷ : A61K 31/00	A2	(11) International Publication Number: WO 00/57860 (43) International Publication Date: 5 October 2000 (05.10.00)
(21) International Application Number: PCT/CA00/00320 (22) International Filing Date: 23 March 2000 (23.03.00) (30) Priority Data: 60/126,376 26 March 1999 (26.03.99) US (71) Applicant (for all designated States except US): CENTRE FOR TRANSLATIONAL RESEARCH IN CANCER [CA/CA]; 3755, chemin de la Côte Ste-Catherine, Suite D-127, Montreal, Québec H3T 1E2 (CA). (72) Inventors; and (75) Inventors/Applicants (for US only): SANDOR, Victor, A. [CA/CA]; 4874 Côte des Neiges, #1508, Montreal, Québec H3V 1H4 (CA). SCRIVENS, Paul, James [CA/CA]; 2134 Dorion, Montreal, Québec H2K 4B2 (CA). (74) Agent: SWABEY OGILVY RENAULT; Suite 1600, 1981 McGill College Avenue, Montréal, Québec H3A 2Y3 (CA).		(81) Designated States: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>Without international search report and to be republished upon receipt of that report.</i>
(54) Title: PEROXOVANADIUM COMPOUNDS AS ANTINEOPLASTIC AGENTS FOR THE TREATMENT OF CANCER (57) Abstract The present invention relates to the use of peroxovanadium compounds as antineoplastic agents for the treatment of cancers, particularly solid tumor malignancies. Further, this invention is directed to the use of modifications to the heteroligand structure of organified peroxovanadium compounds to modulate their activities against specific intracellular targets such as, but not limited to, the CDC 25 family of phosphatases, that may be directly involved in the pathogenesis of cancer.		

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**PEROXOVANADIUM COMPOUNDS AS ANTINEOPLASTIC AGENTS
FOR THE TREATMENT OF CANCER**

BACKGROUND OF THE INVENTION

(a) Field of the Invention

The invention relates to the use of peroxovanadium compounds as antineoplastic agents for the treatment of cancers, particularly solid tumor malignancies. Further, this invention is directed to the use of modifications to the heteroligand structure of organified peroxovanadium compounds to modulate their activities against specific intracellular targets such as, but not limited to, the CDC 25 family of phosphatases, that may be directly involved in the pathogenesis of cancer.

Description of Prior Art

The development of new antineoplastic agents with mechanisms of action that are distinct from the currently available drugs is an important strategy for improving cancer treatment outcomes. While there have been major advances in the chemotherapeutic treatment of malignant diseases, particularly hematological malignancies, most patients with advanced solid tumors will fail to respond or will relapse from their initial response to chemotherapy and ultimately succumb to their disease.

Accordingly, a need exists for a novel class of antineoplastic agents capable of treating cancers resistant to currently available therapeutic options. In addition, it would be highly desirable to modulate the specificity of such agents for specific intracellular targets that may be important for the pathogenesis of cancer by altering an organic heteroligand structure moiety of the agents.

The antineoplastic activity of the vanadium salt (sodium orthovanadate) was reported by Cruz et al.

(*Mol. and Cell. Biochem.*, 1995, **153**:161-166). They demonstrated that at concentrations of 5-10 μ M, sodium orthovanadate is cytotoxic to proliferating cells. They also showed that orthovanadate has *in vivo* cytotoxic activity against a subcutaneous MAYDAY-D2 malignant hematopoietic cell line model in DBA/2j mice. Peroxovanadium compounds represent a significant improvement over these results in that the potency of peroxovanadium compounds is up to 20-fold that of simple vanadium salts *in vitro*. In addition, the potency of peroxovanadium compounds against potential intracellular phosphatase targets is higher and modifications of the heteroligand structure of peroxovanadium compounds are possible to modulate both overall potency and specificity as described in Examples V and VI below. That peroxovanadium compounds with organic heteroligands represent a significant departure from the technology presented by Cruz *et al* is evident from their statement that while peroxovanadate and vanadyl sulfate are more potent inhibitors of tyrosine phosphatases, they are no more potent than orthovanadate as cytotoxic agents.

Surprisingly, the compounds described in this application are more potent, more specific and are amenable to ligand design for targeting to potential phosphatase targets.

As it relates to this application, United States Patent No. 5,693,627 (issued on December 2, 1997) describes the use of a variety of protein tyrosine phosphatase inhibitors including a variety of vanadium compounds for inhibiting the proliferation of B-cell malignancies. The vanadium compound for which data is presented is bis(maltolato)oxovanadium (BMLOV). Peroxovanadium compounds have a broader spectrum of activity than that described for BMLOV in that they

are equally effective against hematological and solid tumor models *in vitro*. In fact United States Patent No. 5,693,627 specifically includes data to show that non-B-cell lines were only moderately inhibited by BMLOV (column 22, lines 39-41) and no claims for the treatment of solid tumors were made. The cytotoxicity data presented in United States Patent No. 5,693,627 uses two different assays which provide two different results. The assay using thymidine incorporation is one most comparable to the method described in this application. Based on this data, peroxovanadium compounds are approximately 2.5-fold more potent than BMLOV. The mechanism of action of peroxovanadium compounds is unclear, however, they are able to induce direct DNA damage (Example IV below) in addition to inhibiting phosphatases (Examples V and VI below) and this may in part account for their broader spectrum of activity and their higher potency than BMLOV.

No *in vivo* efficacy data is presented in United States Patent No. 5,693,627 and thus, it is not possible to comment on differences with regard to *in vivo* efficacy. However, Jackson *et al* used BMLOV to test a polymer-based drug delivery system for antineoplastic agents (*British J. of Cancer*, 1997, **75**(7):1014-1020). They present *in vitro* data of the antineoplastic activity of BMLOV against a variety of cell lines, however, a prolonged exposure of up to 504 hours (21 days) is required to achieve IC50 concentrations of ~10 μ M. Peroxovanadium compounds are up to 50-fold more potent with exposure times of 24 hours (1 day). This *in vivo* data presented by Jackson *et al* is for a prolonged release of the compound from a polymer-based drug delivery system. Systemic administration of BMLOV has activity against the

MAYDAY-D2 model (a B-cell malignancy) in keeping with the claims of United States Patent No. 5,693,627. However, direct local application of the polymere-delivery system is required for activity against a solid tumor model (RIF-1 rat sarcoma). Peroxovanadium compounds have systemic activity against solid tumors (Example II). Thus, the use of peroxovanadium compounds as antineoplastic agents represents a significant improvement over the use of BMLOV in that peroxovanadium compounds are more potent, they require a shorter duration of exposure for their activity, and they are effective systemically against solid tumors. This has significant practical applications for the treatment of metastatic disease where local therapies are ineffective.

It would be highly desirable to be provided with antineoplastic agents for the treatment of cancers, particularly solid tumor malignancies.

It would be highly desirable to be provided with the use of modifications to the heteroligand structure of organified peroxovanadium compounds to modulate their activities against specific intracellular targets such as the CDC 25 family of phosphatases, that may be directly involved in the pathogenesis of cancer.

SUMMARY OF THE INVENTION

One aim of the present invention is to provide antineoplastic agents for the treatment of cancers, particularly solid tumor malignancies.

Another aim of the present invention is to provide the use of modifications to the heteroligand structure of organified peroxovanadium compounds to modulate their activities against specific intracellular targets such as the CDC 25 family of

phosphatases, that may be directly involved in the pathogenesis of cancer.

In accordance with the present invention there is provided a method for treating solid tumor malignancies using peroxovanadium compounds. These compounds are cytotoxic to several human tumor types, and cause cell death through apoptosis. The mechanism of action of these agents has not been fully defined; however, potential mechanisms include inhibition of phosphotyrosine phosphatases and direct DNA damage. By altering the heteroligand structure of the peroxovanadium compounds, the specificity of the compounds for important intracellular targets (such as, but not restricted to the CDC 25 family of cell cycle regulatory phosphatases) can be modulated. The compounds can be administered orally, intravenously, intraperitoneally or subcutaneously. Continuous exposure of cancer cells to adequate concentrations (0.1-100 uM) of the agents for 24-48h is the preferable pharmacokinetic target for the administration regimen.

In accordance with the present invention there is provided the use of a peroxovanadium compound for the preparation of a medicament for the treatment of malignant diseases refractory to other therapies, which comprises a therapeutical effective amount of at least one peroxovanadium compound.

The malignant diseases may include solid tumor malignancies.

In accordance with the present invention there is provided the use of a peroxovanadium compound for the preparation of a medicament for the treatment of drug resistant tumors expressing P-glycoprotein, which comprises a therapeutical effective amount of at least one peroxovanadium compound.

The drug resistant tumors may be cisplatin refractory tumors.

In accordance with the present invention there is provided the use of a peroxovanadium compound for the preparation of a medicament for targeted treatment of malignant diseases refractory to other therapies, which comprises a therapeutical effective amount of at least one peroxovanadium compound specifically targeted to alter heteroligand structure of phosphotyrosine phosphatase.

In accordance with the present invention there is provided a method for the treatment of drug resistant tumors expressing P-glycoprotein in a patient, which comprises administering a therapeutical effective amount of at least one peroxovanadium compound to the patient.

In accordance with the present invention there is provided a method for the targeted treatment of malignant diseases refractory to other therapies, which comprises specifically targeting peroxovanadium compounds against phosphotyrosine phosphatase targets by altering their heteroligand structure.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 illustrates the general structure of peroxovanadium compounds with examples of some possible heteroligand structures.

Fig. 2 illustrates an example of the cytotoxicity profile of 2 different peroxovanadium compounds bisperoxo (4,7-dimethyl-1,10-phenanthroline) oxovanadate [bpVMe2phen] and bisperoxo (1,10-phenanthroline) oxovanadate [bpV(Phen)] on two cell lines MCF-7 (human breast cancer) and SCCA (squamous cell head and neck cancer).

Fig. 3 shows cytotoxicity profiles for bpV(Me2phen) and bpV(Phen) over a series of exposure times, demonstrating that exposures of 24-48 hrs provide optimal cytotoxicity.

Fig. 4 illustrates a photograph of a stained agarose gel after electrophoresis of DNA from cells treated with bpV(Me2phen) showing breakdown of DNA into fragments characteristic of apoptosis.

Fig. 5 illustrates a photograph of an antiphosphotyrosine immunoblot, showing high levels of phosphorylation induced after exposure to as little as 0.7 uM bpV(Me2phen) for 24 hours.

Fig. 6 is a photograph of a stained agarose gel in which whole cells treated with bpV(Me2phen) or cisplatin (a known DNA cross-linking agent) were immobilized in low melting point agarose, lysed, and eletrophoresed under denaturing alkaline conditions. The small smear of stained DNA under the control well is contrasted with the relative lack of migration evident in the wells containing cells treated with either bpV(Me2phen) or cisplatin suggesting that the DNA in these cells is extensively cross-linked.

Fig. 7 shows a graph of the relative ability of various peroxovanadium compounds to inhibit the activity of phosphatases in vitro using an assay with p-nitrophenylphosphate as a chromogenic substrate. The heteroligand structure modulates not only the overall potency of each compound as a phosphatase inhibitor, but also the relative specificity of each compound against different phosphatases. Of note, some peroxovanadium compounds are potent inhibitors of CDC 25A.

Fig. 8 shows *in vivo* data of the ability of daily injections of 15-20 mg/kg of bpV(Me2phen) to inhibit the growth of Lewis Lung Carcinoma cells in C57BL6/j mice.

Fig. 9 shows the inability of peroxovanadium compounds to inhibit alkaline phosphatase where the simple vanadium salt, sodium orthovanadate can inhibit this enzyme, demonstrating a significant difference in the mechanism of inhibition and a relative specificity for protein phosphotyrosine phosphatases for peroxovanadium compounds.

DETAILED DESCRIPTION OF THE INVENTION

In accordance with the present invention, there is provided a method for the use of peroxovanadium compounds as antineoplastic agents for the treatment of cancer, particularly solid tumor malignancies. In addition, the specificity of these compounds against intracellular molecular targets, particularly phosphotyrosine phosphatases such as the CDC 25 family of tyrosine phosphatases can be modulated by alterations in the heteroligand structure.

A method of for the treatment of cancer according to the present invention comprises the step of contacting proliferating cancer cells with peroxovanadium compounds in sufficient quantity as to cause cell death or inhibition of cellular proliferation. This may be achieved by oral, intravenous, intramuscular, topical, subcutaneous, or intraperitoneal administration to achieve a pharmacokinetic profile in which cells are exposed to sufficient quantities of the agent for approximately

24-48 hours. Optimal concentrations and durations of exposure may vary for different compounds and tumor types. The method may also be applied to cells extrocorporeally such as for bone marrow or stem cell purging.

Peroxo vanadium Compounds:

Vanadium has been observed to have a number of biological effects including inhibition of Na^+/K^+ ATPases, inhibition of phosphatases, and stimulation of glucose transport and oxidation through insulin receptor kinase activation. These properties lead to investigations of vanadium in the treatment of diabetes. It was observed that the combination of vanadium salts with hydrogen peroxide was markedly synergistic in mimicking insulin and activating insulin receptor kinases in rat adipocytes. This combination produces a mixture of peroxovanadium complexes in equilibrium with one another. Subsequently, discrete peroxovanadium compounds associated with heteroligands were synthesized and were shown to have strong *in vitro* and *in vivo* insulin mimetic properties (Posner BI et al, *J Biol Chem* , 1994, **269**:4596; Bevan AP et al., *Mol Cell Biochem*, 1995, **153**:49; Shaver A, et al., *Mol Cell Biochem*, 1995, **153**:5). Peroxovanadium compounds are, therefore, a distinct class of organometallic compounds with chemical and biological properties different in many ways from those of simple vanadium salts. The basic structure of peroxovanadium compounds is shown in Fig. 1 (Posner BI et al, *J Biol Chem* , 1994, **269**:4596; Bevan AP et al., *Mol Cell Biochem*, 1995, **153**:49; Shaver A, et al., *Mol Cell Biochem*, 1995, **153**:5). Most peroxovanadium compounds are stabilized with a

bidentate ligand, which forms a 5-membered ring with the vanadium atom. The potency and specificity of these compounds against various phosphatase targets is modulated by the organic heteroligand moiety (Fig. 7). This modulating effect of the heteroligand structure in these compounds allows for extensive ligand design, structure activity correlations and to rational drug design targeting these agents to specific intracellular phosphatase targets which may be important in cancer pathogenesis. Peroxovanadium compounds are stable indefinitely when stored cold in the dark, and those compounds isolated as salts are generally freely soluble in water at neutral pH, thus obviating concerns regarding pharmaceutical formulations³.

The present invention will be more readily understood by referring to the following examples which are given to illustrate the invention rather than to limit its scope.

EXAMPLE I

Peroxovanadium Compounds Have In Vitro Antineoplastic Activity

Peroxovanadium compounds have antineoplastic activity against a large variety of human tumor cell lines. Table 1 shows the growth inhibitory effects determined using the standard sulforhodamine B assay used by the NCI anticancer drug screen and as described by Skehan *et al* of 5 different peroxovanadium compounds against 3 human tumor cell lines: MCF-7 (breast); SW620 (colon); and SSCA (head and neck) (Skehan P *et al.*, *J Natl Cancer Inst*, 1990, **82**:1107).

Table 1

Cell line	Compound				
	Phen	Me2Phen	BiPy	BiPy-(CO ₂) ₂	Pic
SCC-A	1.1	0.3	3.5	13.0	3.5
SW620	2.6	0.7	6.5	27.0	6.5
MCF-7	2.6	0.6	9.5	60.0	15.0
L/L2	1.2	0.3			

The cytotoxicity profile of each peroxovanadium compound is clearly modulated by the organic heteroligand structure. The two most active compounds among these 5 tested were bpV(phen) and bpV(Me2phen), with IC₅₀ concentrations in the submicromolar and low micromolar range. These compounds were subsequently screened against 20 cell lines representing several solid tumor and leukemia cell lines from the NCI anticancer drug screen. The IC₅₀ values for these two compounds in each of the cell lines tested is shown in Table 2.

Table 2

	Phen	Me2Phen		Phen	Me2Phen
Prostate			Renal		
PC 3	2.24	0.35	786-0	1.12	0.63
DU 145	1.24	0.28	A498	1.05	0.75
Non-Small Cell Lung			ACHN	1.80	0.37
A549	1.80	0.37	UO 31	-	0.27
EKVx	1.40	1.05	Breast		
Colon			MCF-7	2.95	0.84
SW 620	2.61	0.78	NIHADR	1.23	0.24
CNS			MB 231	-	0.42
SF 295	-	0.74	MB 468	-	0.62
SNB 75	7.50	2.00	Leukemia		
Ovarian			Cem	2.75	0.80
OVCAR 3	2.00	0.84	Molt 4	1.95	0.50
OVCAR 4	2.70	0.31	K562	1.22	0.32

Of note, the most sensitive cell line is the drug resistant NIH ADR breast cancer cell line, suggesting that these agents may be particularly active against relatively drug resistant tumors.

EXAMPLE II

Peroxovanadium compounds have significant in vivo antineoplastic activity

Six test and six control animals were injected subcutaneously on the left flank with 10^6 L/L2 cells. Test animals were given daily intraperitoneal injections of 18 mg/Kg of bpV(Me2phen) starting two days after tumour implantation. The tumours were measured bidimensionally over time in both test and control groups. Tumour volumes were estimated using the formula: Tumor volume = $(\text{Length} \times \text{Width}^2)/2$ (where the width represents the smaller of the bidimensional measurements). Figure 8 shows a graphical representation of the growth of the tumours over time. The compound showed some toxicity including lethargy, fatigue and weight loss, but histological examination of lung, liver and kidney as well as bone marrow cytology after the animals were sacrificed, showed no evidence of end organ damage. These results show a significant antitumour activity of bpV(Me2phen) in this solid tumour model.

EXAMPLE III

Peroxovanadium Compounds Induce Apoptotic Cell Death

While the exact molecular mechanism of action of peroxovanadium compounds remains unknown, an antineoplastic action of these agents is through the induction of apoptotic cell death. Fig. 4 shows

internucleosomal DNA laddering characteristic of apoptotic cell death caused by bpV(Me2phen) in SW620 colon cancer cells.

EXAMPLE IV

Potential Mechanisms of Antineoplastic Action

The mechanism of antineoplastic action of peroxovanadium compounds is not clearly understood and may be multifactorial.

Phosphatase inhibition

Peroxovanadium compounds are potent protein phosphotyrosine phosphatase inhibitors, and these phosphatases are important in the regulation of signal transduction and cell cycle regulation-pathways often disrupted in the pathogenesis of cancer. For example, the human CDC25 dual specificity phosphatases trigger activation of cyclin dependant kinases (CDK's) by removing inhibitory phosphates from tyrosine and threonine residues (Berry LD, Gould KL., *Prog Cell Cycle Res*, 1996, 2:99). Genes encoding some of these phosphatases have been identified as potential oncogenes both *in vitro* and in various solid tumor malignancies including breast cancer, gastric cancer, head and neck cancer, lung cancer as well as some lymphomas. Peroxovanadium compounds are able to alter the level of cellular tyrosine phosphorylation at concentrations required for antineoplastic activity. Fig. 5 shows an antiphosphotyrosine immunoblot after standard SDS-PAGE of lysates from cells treated with bpV(Me2phen). Treatment with 0.7 uM bpV(Me2phen) for 24 hrs causes a dramatic increase in cellular tyrosine

phosphorylation which may in part account for the cytotoxicity of this compound.

Cell cycle disruption

The human CDC25 dual specificity phosphatases trigger activation of cyclin dependant kinases (CDK's) by removing inhibitory phosphates from tyrosine and threonine residues. Genes encoding these phosphatases are potential oncogenes because of their role in promoting cell division. Three human CDC25 genes have been identified: *CDC25A*, *CDC25B*, and *CDC25C*. *CDC25A* has been mapped to chromosome 3p21 by fluorescence in situ hybridization with confirmation by PCR analysis of hamster/human somatic cell hybrid DNAs. An area near 3p21 is frequently involved in karyotypic abnormalities in renal carcinomas, small cell carcinomas of the lung, and benign tumors of the salivary gland. Galaktionov et al showed that in rodent cells, transformation with human *CDC25A* or *CDC25B* but not *CDC25C* cooperate with mutations of the H-RAS gene or loss of RB1 in oncogenic focus formation. The transformants were highly aneuploid, grew in soft agar, and formed high grade tumors in nude mice. Thus, *CDC25* isoforms are human oncogenes. In addition, over expression of *CDC25B* was detected in 32% of samples from 124 primary breast cancers tested by these investigators. Other investigators have detected over-expression of *CDC25* in gastric carcinomas (*CDC25B*), head and neck carcinomas (*CDC25A* and *CDC25B*), and in non-Hodgkin's lymphomas, where *CDC25B* over-expression was highly correlated with aggressive histologies including Burkitt's lymphoma.

The c-MYC proto-oncogene has been shown to play a role in the pathogenesis of several different human

tumors including leukemias, lymphomas, gliomas as well as breast, stomach, lung and colon cancers. The c-MYC oncoprotein, in partnership with MAX, forms a transcription factor that can promote oncogenic transformation. Galaktionov et al have shown that the MYC/MAX heterodimer binds to elements in the *CDC25A* gene and activates its transcription. They also showed that *CDC25A* is required for c-MYC induced transformation and apoptosis. Steiner et al, using density arrested fibroblasts showed that CDK activation and cell cycle progression by c-MYC requires intact DNA binding and heterodimerization domains, and is blocked by inhibitors of transcription. They identified a c-MYC dependent and a *CDC25A* dependent step in the process of CDK activation. Taken together with the data from Galaktionov et al, *CDC25A* is a transcriptional target of c-MYC and it plays an important role as a mediator of c-MYC functions.

The *CDC25* family of phosphatases are key regulators of cell cycle progression and, therefore, their inhibition would be expected to be antiproliferative and potentially cytotoxic. In addition, *CDC25* isoforms may be important either directly or through c-MYC in the pathogenesis of several different tumor types, thus making them rational targets for antineoplastic drug development.

MCF-7 cells treated with 0.5, 0.75 and 1.0 μ M bpV(me2phen) were lysed, and the *CDC25A* substrate, cyclin dependant kinase 2 (CDK-2), was immunoprecipitated using a specific polyclonal antibody (provided by Dr. A. Senderowics, NIH, NCI). The immunoprecipitates were then used in a kinase assay to

determine the activity of CDK-2. Histone H1 was used as a substrate, and kinase activity was determined by measuring the incorporation of P^{32} from P^{32} γ -ATP using SDS-PAGE followed by autoradiography and then densitometry. As CDC25A is required for the activation of CDK-2, if bpV(me2phen) inhibits CDC25A, one would predict a decrease in CDK-2 activity. Figure 6 shows a representative autoradiograph along with the densitometry results normalized to control and shown in a graphical format. A dose dependent decrease in CDK-2 activity is observed.

The retinoblastoma protein (Rb) plays a central role in regulation of the transition from G₀ through G₁ and into the S-phase of the cell cycle. In its unphosphorylated form, Rb binds to a family of transcription factors, the E2Fs. These complexes of Rb and E2F act as transcriptional repressors and serve to sequester the active transcription factor, E2F. Activated CDK-2 phosphorylates Rb and causes it to release E2F thus, promoting G₁-to-S transition. Figure 6 shows the results of an experiment in which the immortalized breast cell line MCF-10A is arrested in G₀ by serum starvation for 24 hours. The cells are then restimulated to enter the cell cycle by the addition of serum and epidermal growth factor (EGF). Cell lysates are prepared at time points after the addition of serum in the presence or absence of bpV(me2phen). The lysates are then separated by SDS-PAGE and immunoblotted using an antibody specific for phosphorylated Rb. At the 29 hour time point, phospho-Rb is detected in the control cells, but no

phosphorylation of Rb occurs in cells treated with bpV(me2phen). This result is consistent with the inhibition of CDK-2 activity demonstrated in Figure 6.

EXAMPLE V

The Specificity of Peroxovanadium Compounds for Phosphatases is Modulated by the Heteroligand

The ability of a panel of pV compounds to inhibit the phosphatase activity of three different protein tyrosine phosphatases was assayed. T-cell and leukocyte antigen receptor (LAR) phosphatases were obtained from CalBiochem. CDC25A phosphatase was produced in my laboratory as a GST-fusion protein from a plasmid provided by Dr. K. Galaktionov, Baylor Medical College. The purity of the preparation was confirmed by SDS-PAGE and direct staining as well as by immunoblotting using a CDC25A specific antibody (SantaCruz). Para-nitrophenyl phosphate (pNPP) was used as a colorimetric substrate for these experiments. Figure 7 shows the relative activity of each of seven pV compounds against these three phosphatases. Enzyme inhibition occurs in the nM range (15-170 nM). Interestingly, IC50 values for direct enzyme inhibition are approximately 100-fold lower than IC50 values determined for cytotoxicity (see Tables 1 and 2). This intra-to-extracellular gradient is likely attainable and is consistent with the idea of phosphatase inhibition as a mechanism of action for these compounds. Also of note is the fact that GST-CDC25A is generally more sensitive to pV inhibition than either LAR or T-cell phosphatases by a factor of up to 3-fold. The specificity of pV compounds against protein

tyrosine phosphatases is suggested in Figure 7. Figure 9 shows the activity of these pV compounds against alkaline phosphatase, a phosphatase with a different mechanism of action than protein tyrosine phosphatases. Concentrations of up to 1mM of pV show no significant enzyme inhibition. The specificity of pV compounds relative to simple vanadium salts is also demonstrated in that sodium metavanadate causes significant inhibition of alkaline phosphatase at between 0.25mM and 1mM.

EXAMPLE VI

Peroxovanadium Compounds are Mechanistically Different from Vanadium Salts

Fig. 9 shows the relative inactivity of several peroxovanadium compounds as inhibitors of alkaline phosphatase at concentrations as high as 1mM. Alkaline phosphatase is an enzyme that is structurally and mechanistically different from protein tyrosine phosphatases. In contrast, the vanadium salt sodium orthovanadate is able to inhibit alkaline phosphatase significantly at this concentration, suggesting a relative specificity of peroxovanadium compounds against protein tyrosine phosphatases. These data also suggest a significant mechanistic difference between simple vanadium salts and peroxovanadium compounds which may account for the enhanced antineoplastic potency of peroxovanadium compounds.

ADVANTAGES OF THE PRESENT INVENTION

The present invention provides a method for the use of peroxovanadium compounds as antineoplastic agents for the treatment of cancer. This method

provides a novel agent for the treatment of malignancies that does not show cross-resistance to several currently available treatments. Peroxovanadium compounds have significant advantages over other vanadium compounds or simple vanadium salts in that the concentrations required for activity are significantly lower than that required for other agents, that the demonstrated spectrum of activity of these compounds includes a wide range of solid tumor malignancies as well as hematological malignancies, that the mechanism of action may involve inhibition of several cellular functions including phosphatase inhibition but also direct DNA damage or other mechanisms and that the heteroligand structure of these compounds can be modified to modulate not only overall potency but also patterns of specificity.

While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention and including such departures from the present disclosure as come within known or customary practices within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth, and as follows the scope of the appended claims.

WHAT IS CLAIMED IS:

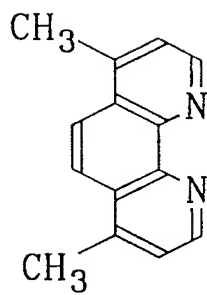
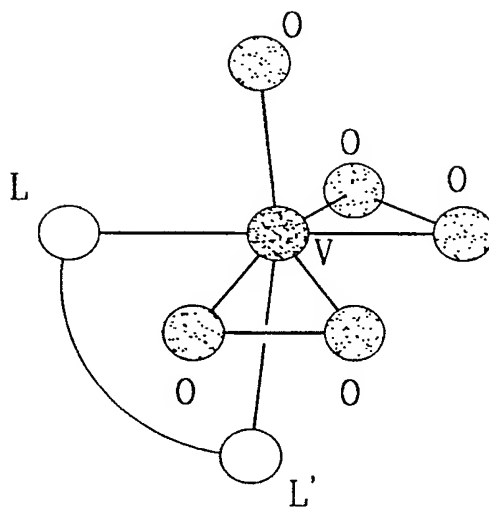
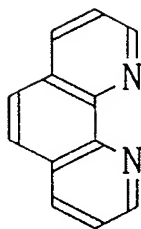
1. The use of a peroxovanadium compound for the preparation of a medicament for the treatment of malignant diseases refractory to other therapies, which comprises a therapeutical effective amount of at least one peroxovanadium compound.
2. The use of claim 1, wherein said malignant diseases include solid tumor malignancies.
3. The use of a peroxovanadium compound for the preparation of a medicament for the treatment of drug resistant tumors expressing P-glycoprotein, which comprises a therapeutical effective amount of at least one peroxovanadium compound.
4. The use of claim 3, wherein said drug resistant tumors are cisplatin refractory tumors.
5. The use of a peroxovanadium compound for the preparation of a medicament for targeted treatment of malignant diseases refractory to other therapies, which comprises a therapeutical effective amount of at least one peroxovanadium compound specifically targeted to alter heteroligand structure of phosphotyrosine phosphatase.
6. A method for the treatment of drug resistant tumors expressing P-glycoprotein in a patient, which comprises administering a therapeutical effective

amount of at least one peroxovanadium compound to said patient.

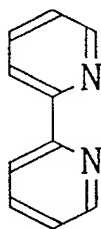
7. The method of claim 6, wherein said drug resistant tumors are cisplatin refractory tumors.

8. A method for the targeted treatment of malignant diseases refractory to other therapies, which comprises specifically targeting peroxovanadium compounds against phosphotyrosine phosphatase targets by altering their heteroligand structure.

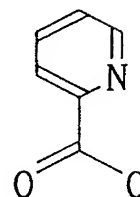
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4,7-Me₂phen

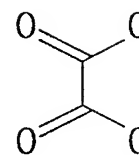
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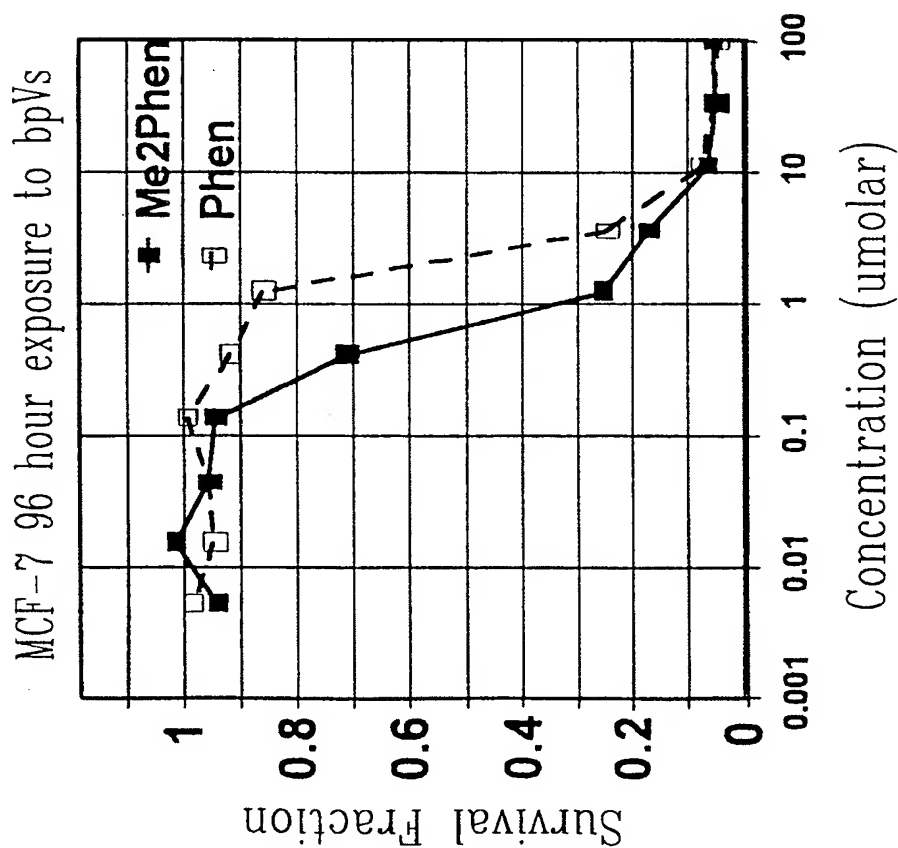
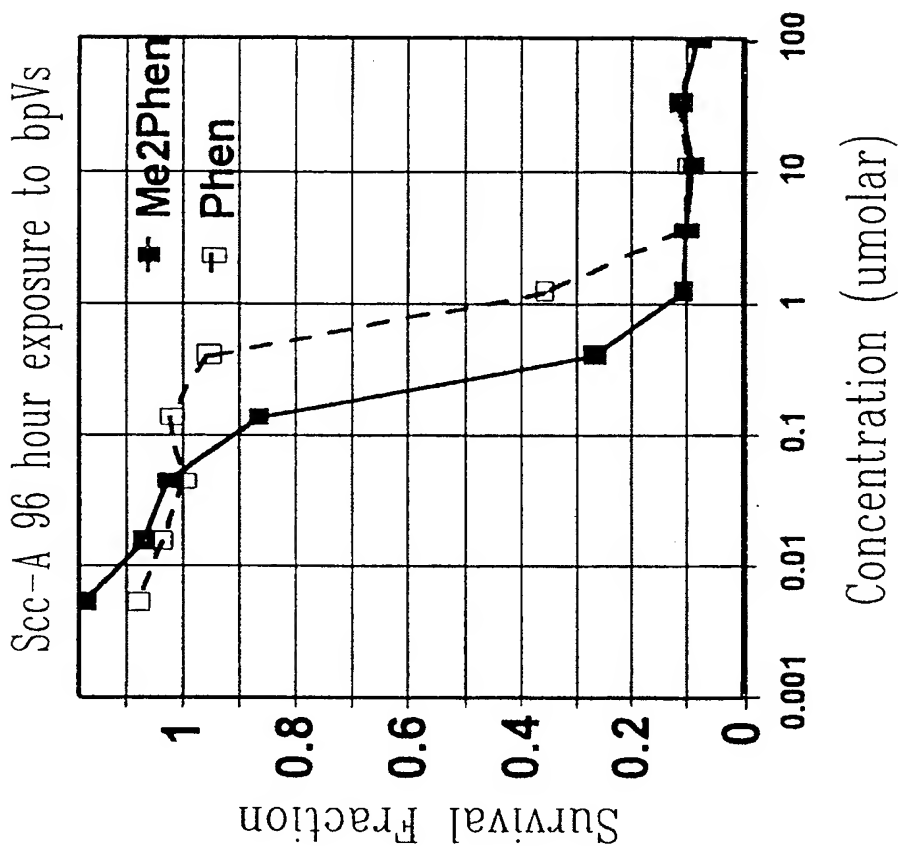
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FIG. 1

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SUBSTITUTE SHEET (RULE 26)

FIG - 2B

FIG - 2A

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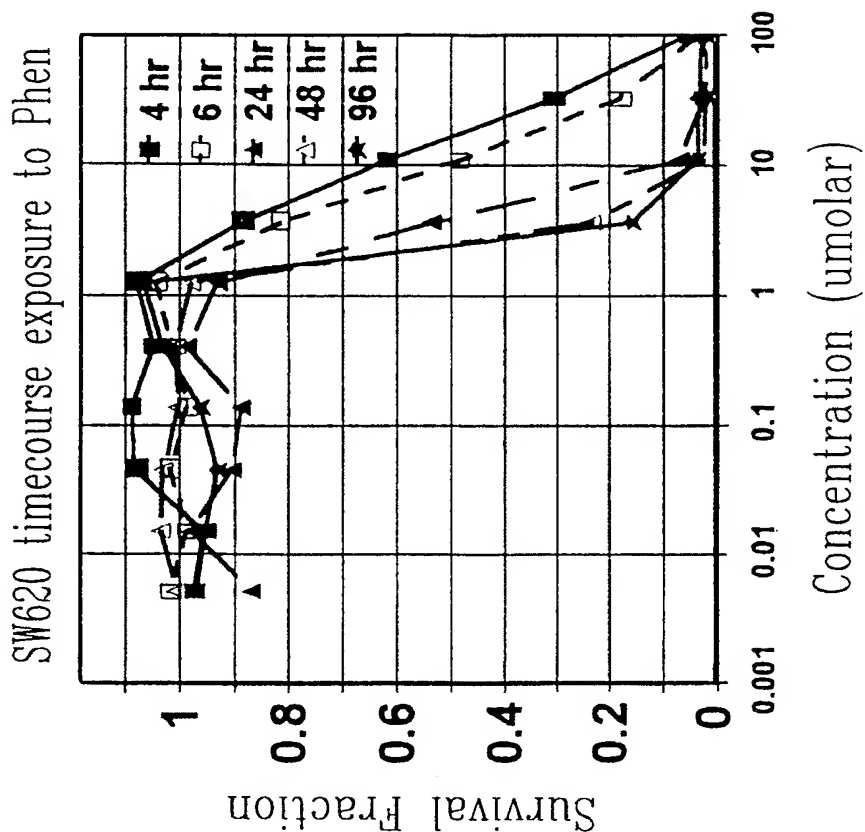


FIG - 3B

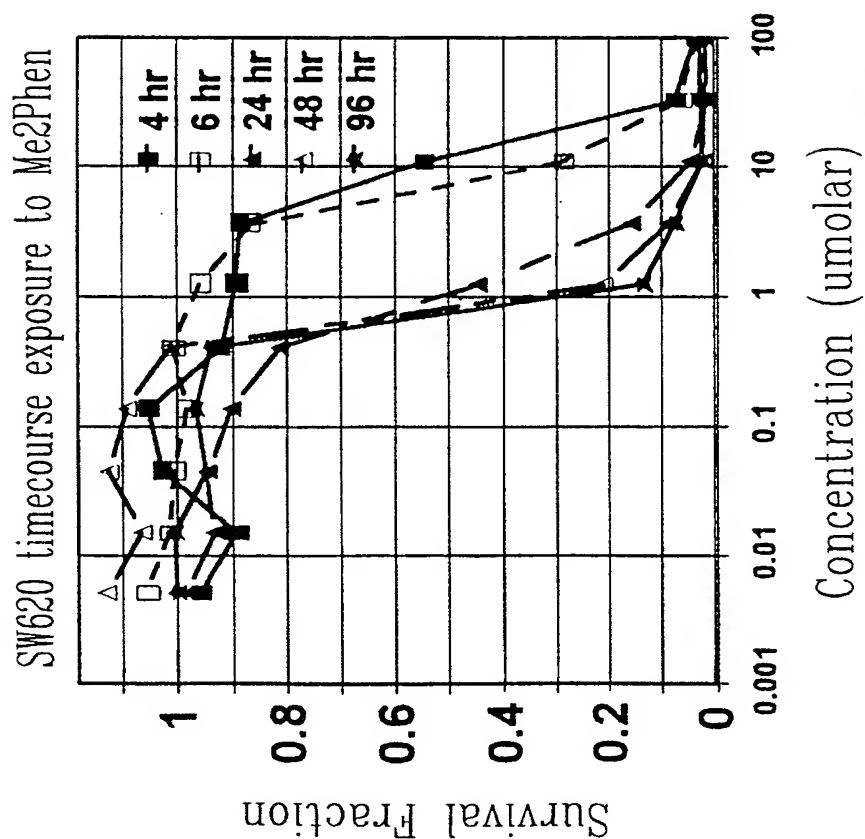


FIG - 3A

SUBSTITUTE SHEET (RULE 26)

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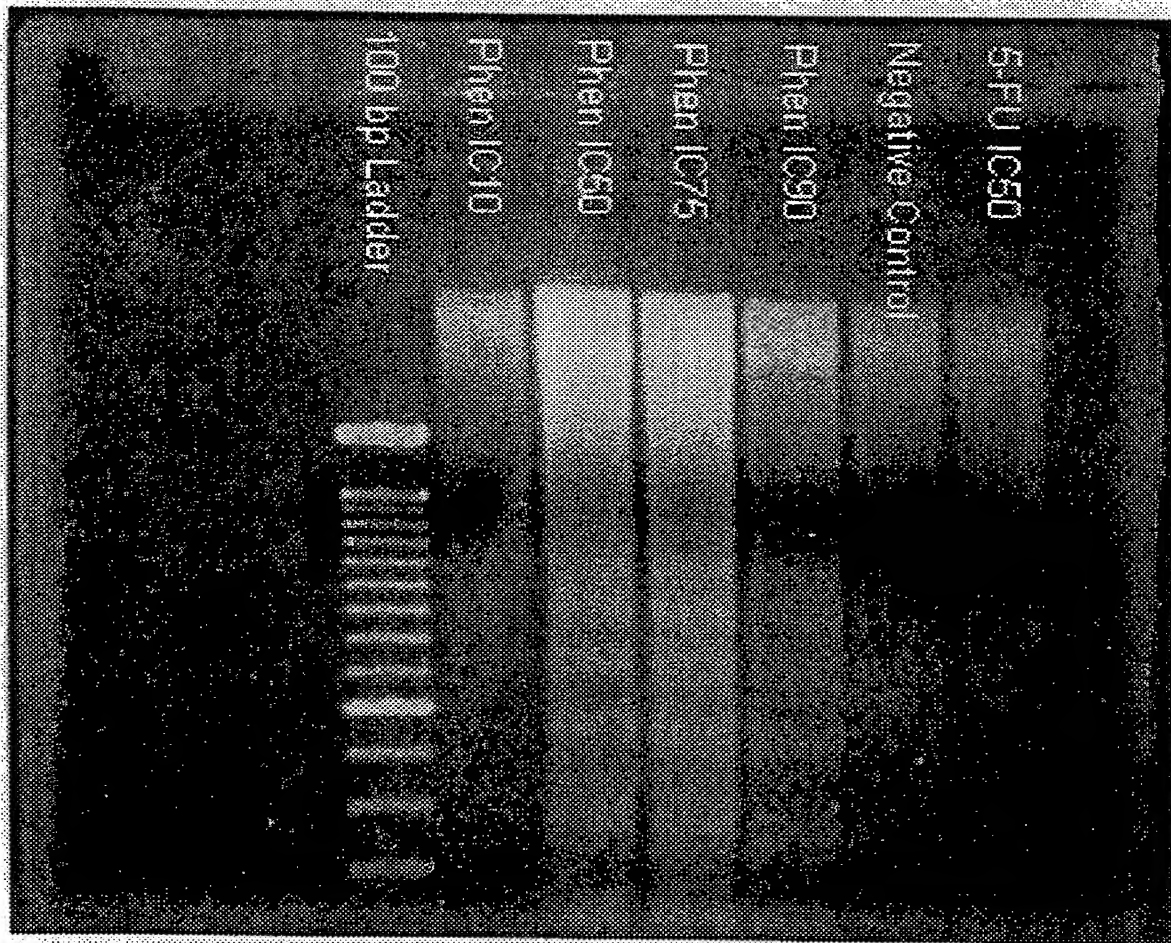


FIG. 4

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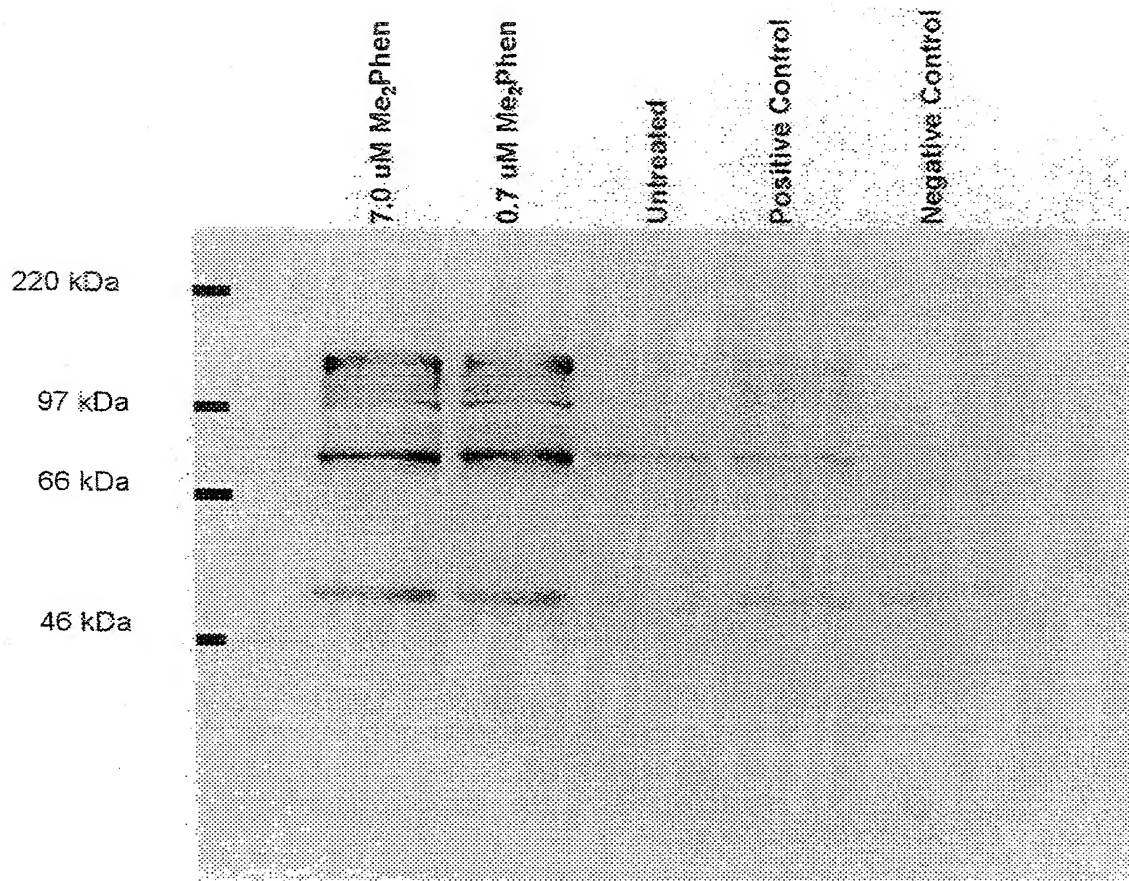
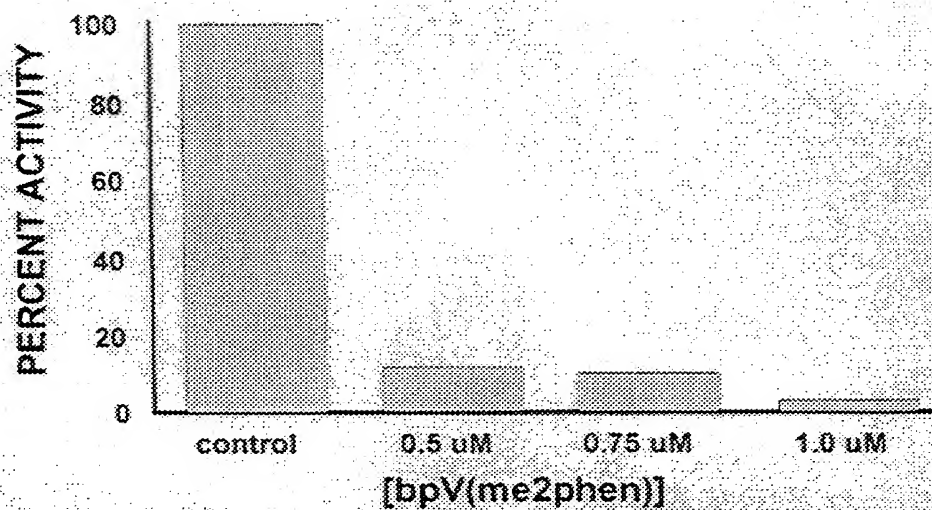
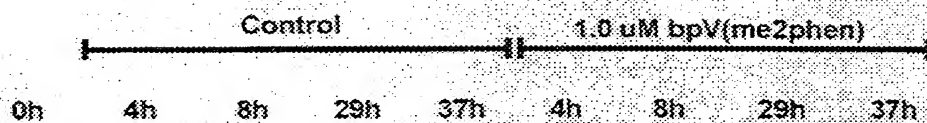


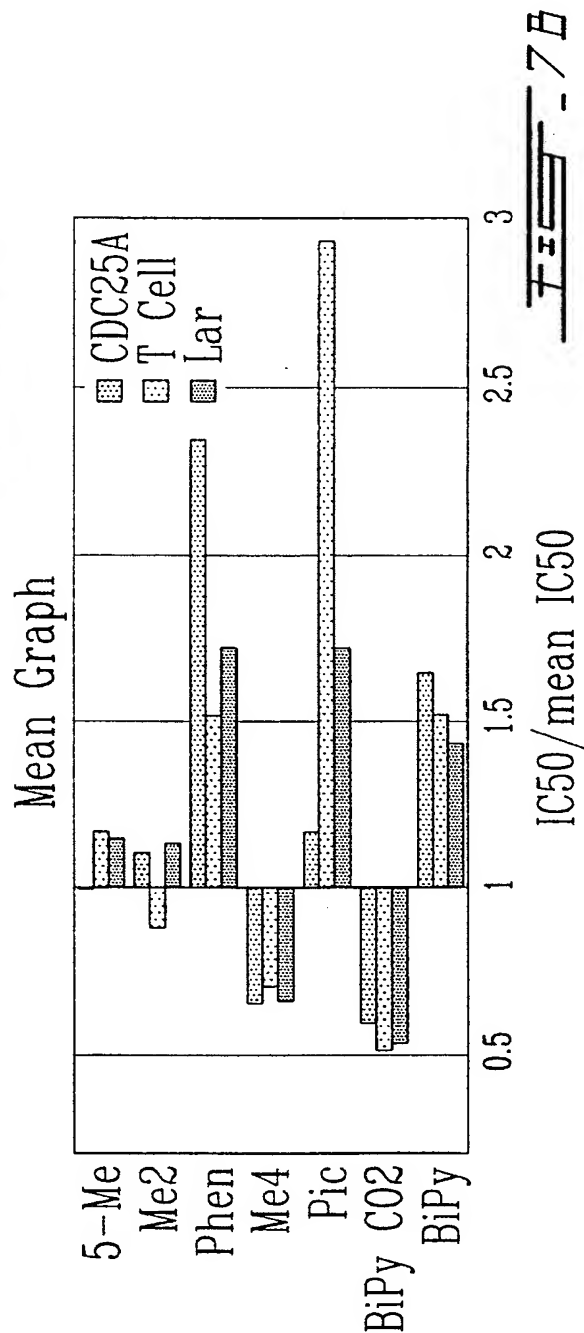
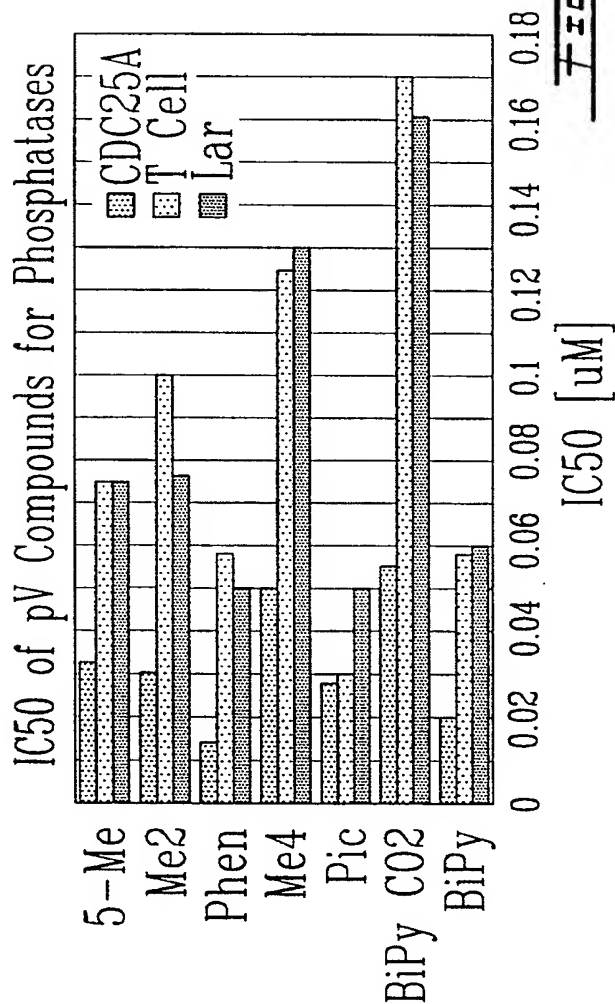
FIG. 5

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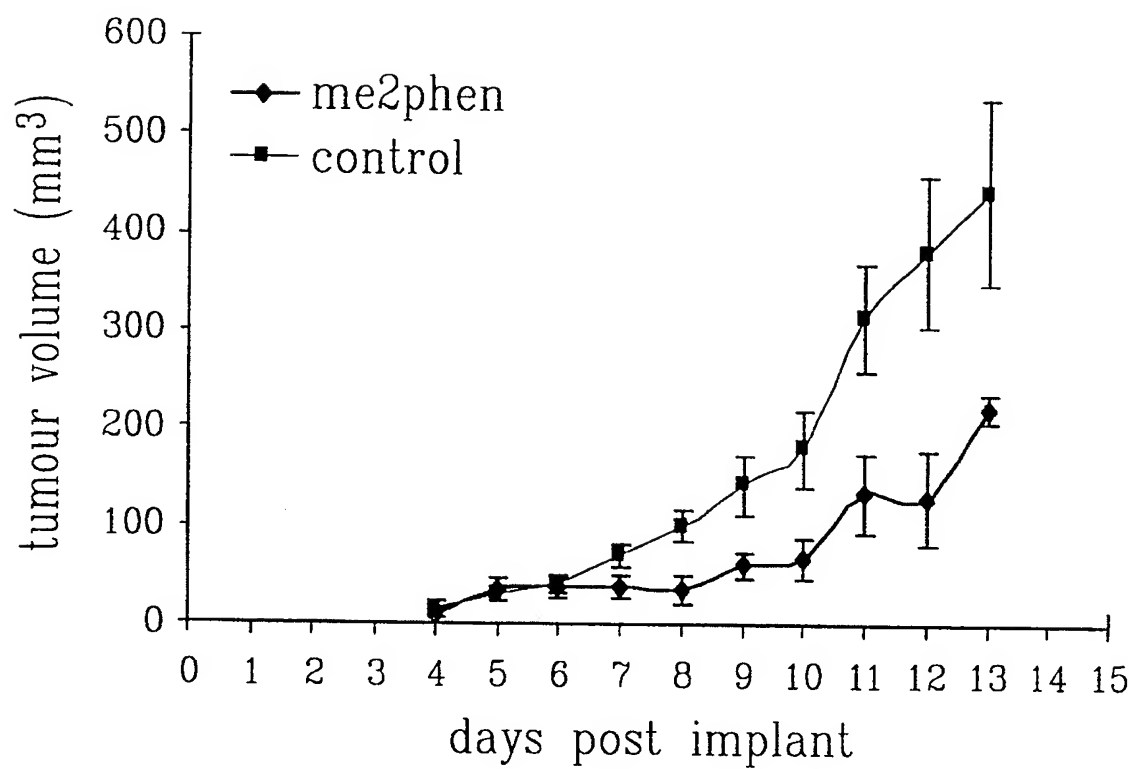
**P³² Histone H1**

← RbpSer795

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FIG. 8

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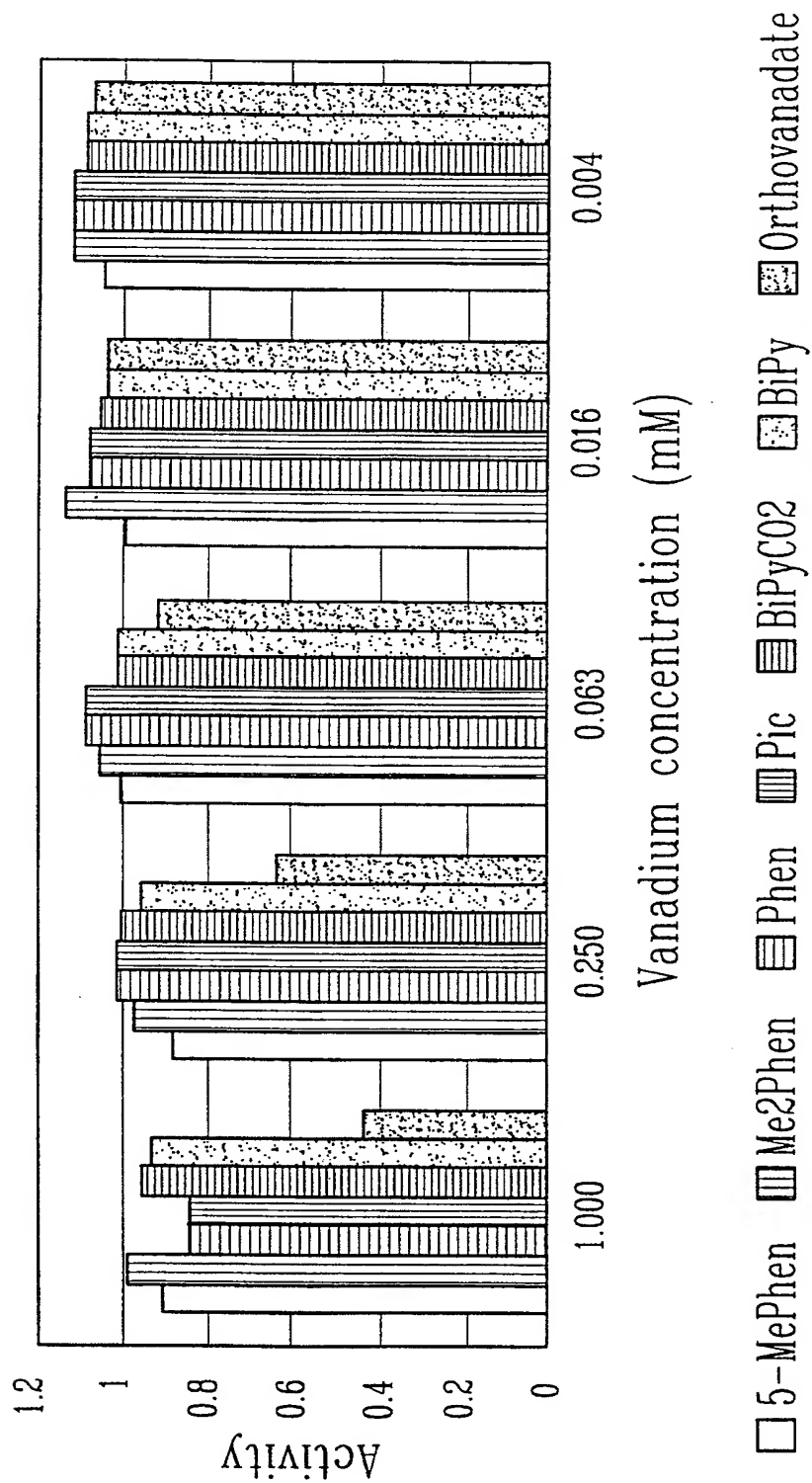


FIG. 9